

Fatty acids affect proliferation of peripheral blood mononuclear cells in dairy cows

N. Lacetera, G. Kuzminsky, P. Morera, L. Basiricò

Dipartimento di Produzioni Animali. Università della Tuscia, Viterbo, Italy

Corresponding author: Nicola Lacetera. Dipartimento di Produzioni Animali. Facoltà di Agraria, Università della Tuscia. Via San Camillo de Lellis, 01100 Viterbo, Italy - Tel. +39 0761 357441 - Fax: +39 0761 357434 - Email: nicgio@unitus.it

ABSTRACT: In vitro studies were performed to assess the effects of bovine plasma fatty acids on proliferation of peripheral blood mononuclear cells (PBMC). PBMC from 6 Holstein heifers were cultured in media containing oleic (OA), palmitic (PA), stearic (SA), linoleic (LA), palmitoleic (POA), or linolenic (LNA) acid at concentrations mimicking different degree of lipomobilisation. Proliferation of PBMC was stimulated by concanavalin A or pokeweed mitogen. Concentrations of OA, PA, SA and LA mimicking moderate-intense lipomobilisation impaired PBMC proliferation. Concentrations of OA or LA mimicking low degree of lipomobilisation enhanced PBMC proliferation. None of the POA, and LNA concentrations affected proliferation of PBMC.

Key words: Dairy cows, Peripheral blood mononuclear cells, Fatty acids.

INTRODUCTION – Intense lipomobilisation may alter reproductive (Leroy *et al.*, 2005) and immune functions (Lacetera *et al.*, 2005) in dairy cows. Hammon *et al.* (2006) showed that intense lipomobilisation is associated with high incidence of metritis corroborating the hypothesis that high concentrations of plasma fatty acids (FA) are associated with immunosuppression in dairy cows. A previous study demonstrated that only some of the FA represented in plasma affect lymphocyte functions in sheep (Lacetera *et al.*, 2002). Present study was aimed to ascertain which FA represented in bovine plasma affect peripheral blood mononuclear cells (PBMC) proliferation at concentrations mimicking those of cows undergoing different degree of lipomobilisation.

MATERIAL AND METHODS – Six healthy and early pregnant Holstein heifers were used as blood donors. Blood samples were collected via jugular venipuncture, using evacuated glass tubes coated with sodium heparin (10 U of heparin/ml). PBMC were isolated by density gradient centrifugation. Blood diluted in phosphate buffer saline (PBS) was layered over Ficoll-Paque PLUS and centrifuged (600 x *g* for 45 minutes at 18°C). The mononuclear cell band was recovered and washed twice in PBS using centrifugation (400 x *g* for 10 minutes at 4°C). Residual red blood cells were lysed by hypotonic shock treatment using redistilled water. The PBMC recovery and viability were determined by hemocytometer count using the trypan blue exclusion method. Viability of PBMC typically exceeded 90%. After isolation, PBMC were resuspended at a concentration of 1 x 10⁶ cells/ml of RPMI-1640 enriched culture medium (ECM). The ECM was represented by RPMI-1640 containing 25 mM HEPES, 10% heat-inactivated foetal bovine serum, 2 mM L-glutamine, 100 U of penicillin, 100 µg of streptomycin, and 0.25 µg of amphotericin B/ml. Triplicate cultures were assayed, by using 96-well tissue-culture plates. Each well contained 1 x 10⁵ mononuclear cells in 100 µl of ECM. Control wells contained 100 µl of PBMC suspension without mitogens. Additional control wells were used that contained 100 µl of ECM without cells, 100 µl of PBMC suspension without the pyrimidine analogue 5-bromo-2'-deoxyuridine (BrdU), or 100 µl of PBMC with or without mitogens in the presence of 0.1% ethanol in the culture media (see below). An optimal concentration of concanavalin A (ConA, 2.5 µg/ml) or pokeweed mitogen (PWM, 1 µg/ml) was added to plates. Before the incubation, plates were added with solutions of oleic (OA, 615, 410, 205, 102, 51 or 26 µmol/L), palmitic (PA, 371, 247, 124, 62, 31 or 15 µmol/L), stearic (SA, 334, 223, 111, 56, 28 or 14 µmol/L), linoleic (LA, 103, 69, 34, 17, 9 or 4 µmol/L), palmitoleic (POA, 49, 32, 16, 8, 4 or 2 µmol/L), or linolenic (LNA, 30, 20, 10, 5, 2 or 1 µmol/L) acid. FA were purchased from Sigma (Milan, Italy). Concentrations tested were designed to mimic those of cows undergoing different degree of lipomobilisation. The solutions of FA were prepared by dissolving each fatty acid in ethanol. Final concentration of ethanol in the culture media did not exceed 0.1%, and was not toxic to the cells. Plates were thus incubated in an atmosphere of 95% air and 5% CO₂ for 48 h at 39 °C. Afterwards, 100 µM BrdU in 10 µl of RPMI-1640 were added to each well, and plates were incubated for an addi-

tional 18 h. The DNA synthesis was quantified by an ELISA assay. The assay was performed with a commercial kit (Amersham Biosciences, Milan, Italy) that is based on measurement of BrdU incorporated during DNA synthesis in proliferating cells. Values for DNA synthesis were expressed as the optical density (OD) for test wells minus the OD for control wells that did not contain BrdU. Intra-assay coefficient of variation was 4.8%. Data were analysed by a one way analysis of variance, and the differences were considered to be significant at $P < 0.05$.

RESULTS AND CONCLUSIONS – High concentrations of OA (615, 410, or 205 $\mu\text{mol/L}$) inhibited (P ranging from < 0.05 and < 0.001) DNA synthesis of ConA- and PWM-stimulated PBMC (Figure 1). Conversely, addition of low concentrations of OA (51 or 26 $\mu\text{mol/L}$) to PBMC cultures enhanced ($P < 0.001$) DNA synthesis of PWM-stimulated cells. Addition of PA (371, 247, 124, 62, or 31 $\mu\text{mol/L}$) to the culture media inhibited (P ranging from < 0.05 and < 0.001) DNA synthesis of mitogen-stimulated PBMC (Figure 2). The higher concentrations of SA (334, 223, 111, or 56 $\mu\text{mol/L}$) inhibited (P ranging from < 0.05 and < 0.01) the DNA synthesis of mitogen-stimulated PBMC (Figure 3). The highest concentration of LA (103 $\mu\text{mol/L}$) inhibited (P ranging from < 0.05 and 0.01) DNA synthesis of ConA- and PWM-stimulated PBMC (Figure 4). Conversely, addition of LA to the culture media at concentration of 9 $\mu\text{mol/L}$ enhanced ($P < 0.01$) DNA synthesis of PWM-stimulated cells. None of the concentrations of POA or LNA affected DNA synthesis of mitogen-stimulated PBMC (data not shown).

In summary, concentrations of OA, PA, SA and LA mimicking moderate-intense lipomobilisation impaired PBMC proliferation, whereas concentrations of OA or LA mimicking low degree of lipid mobilisation exerted some stimulatory effects. These results indicate that FA of bovine plasma interfere with PBMC proliferation in a dose-dependent manner, and that the effects depend on type and concentration of each fatty acid, because the same concentrations of several FA exerted disparate effects (inhibition, stimulation, no effect). Finally, present results reinforce the

Figure 1. Effects of oleic acid on proliferation of peripheral blood mononuclear cells (PBMC) stimulated with concanavalin A (ConA, (A)) or pokeweed mitogen (PWM, (B)). Values are mean \pm SEM. Value assigned for the control group was 100%. Values with asterisks differ significantly from value for control group (* $P < 0.05$, ** $P < 0.001$).

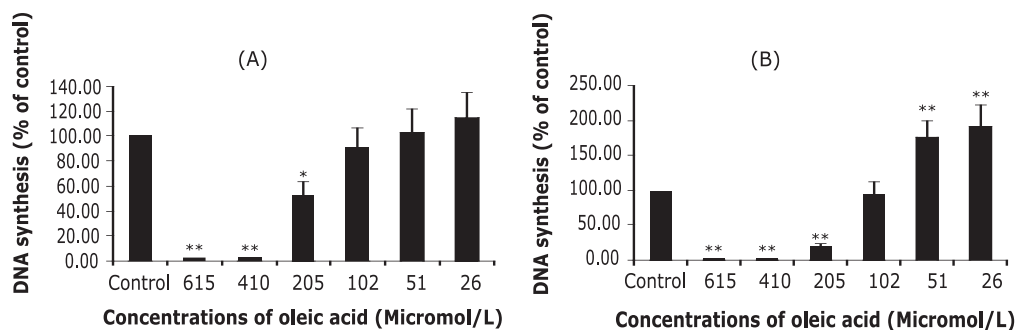


Figure 2. Effects of palmitic acid on proliferation of PBMC stimulated with ConA (A) or PWM (B). Values are mean \pm SEM. Value assigned for the control group was 100%. Values with asterisks differ significantly from value for control group (* $P < 0.05$, ** $P < 0.001$).

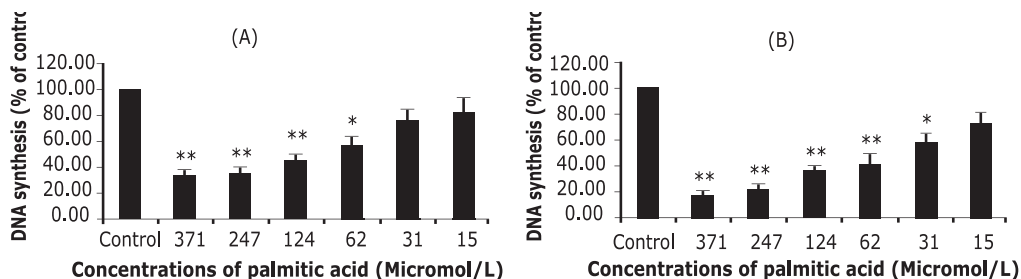


Figure 3. Effects of stearic acid on proliferation of PBMC stimulated with ConA (A) or PWM (B). Values are mean \pm SEM. Value assigned for the control group was 100%. *Values with asterisk differ significantly ($P < 0.01$) from value for control group.

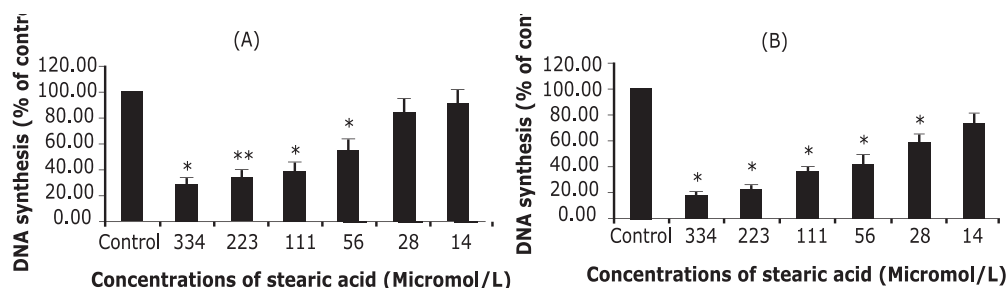
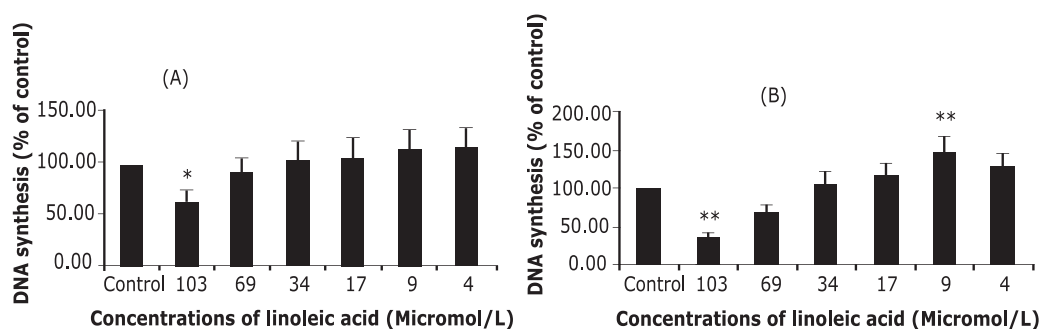


Figure 4. Effects of linoleic acid on proliferation of PBMC stimulated with ConA (A) or PWM (B). Values are mean \pm SEM. Value assigned for the control group was 100%. Values with asterisks differ significantly from value for control group (* $P < 0.05$, ** $P < 0.01$).



hypothesis that lipid mobilisation plays a role in the immunosuppression taking place in transition dairy cows, and that FA are likely to contribute to regulation of the immune response by participating in establishing the equilibrium between up- and down-regulation. Further in vitro studies are being carried out to ascertain the effects of different FA mixture on PBMC functions in dairy cows.

The research was supported by MIUR (PRIN, 2005) and Università della Tuscia.

REFERENCES – **Hammon, D.S.**, Evjen, I.M., Dhiman, T.R., Goff, J.P., Walters, J.L., 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. *Vet. Immunol. Immunopathol.* 113:21-29. **Lacetera, N.**, Franci, O., Scalia, D., Bernabucci, U., Ronchi, B., Nardone, A., 2002. Effects on functions of ovine blood mononuclear cells for each of several fatty acids at concentrations found in plasma of healthy and ketotic ewes. *Am. J. Vet. Res.* 63:958-962. **Lacetera, N.**, Scalia, D., Bernabucci, U., Ronchi, B., Pirazzi, D., Nardone, A. 2005. Lymphocyte functions in overconditioned cows around parturition. *J. Dairy Sci.* 88:2010-2016. **Leroy, J.L.M.**, Vanholder, T., Mateusen, B., Christophe, A., Opsomer, G., de Kruif, A., Genicot, G., Van Soom, A., 2005. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. *Reproduction* 130:485-495.